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Vet., Microbiol., 12:255-268, 1986.

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Results of a Preliminary Trial with Sphaerophorus necrophorus Toxoids to Control Liver Abscesses in Feedlot Cattle

M. M. Garcia, W. J. Dorward, D.C. Alexander, S. E. Magwood and K. A. McKay*

ABSTRACT

A preliminary field experiment was undertaken to evaluate the efficacy of alum precipitated toxoids of Sphaerophorus necrophorus prepared from sonicated whole cells and cell fractions to reduce the incidence of bovine abscesses. A total of 108 calves were divided into five groups and treated as follows: I. uninoculated control, II. adjuvant inoculated control, III. 15.5 mg protein of sonicated (fragmented cells) toxoid, IV. 10.5 mg protein of cytoplasmic toxoid, and V. 15.5 mg protein of cytoplasmic toxoid. All animals were maintained under similar conditions to those prevailing in feedlots in Alberta. Livers were examined at slaughter. The most promising result was achieved with the injection of 15.5 mg protein of cytoplasmic toxoid. In this treatment group, no scars (healed lesions) were found in the liver and the incidence of liver abscesses was reduced to 10% from the average 35% liver abscesses and scars found in the uninoculated and adjuvant inoculated groups. The toxoid from sonicated whole cells did not reduce liver abscess incidence. These data suggest that the incidence of liver abscesses in cattle fattened in feedlots may be reduced by immunization.

RÉSUMÉ

On a réalisé, sur une ferme, une première expérience visant à évaluer l'efficacité de toxoïdes de Sphaerophorus necrophorus précipitées à l'alun et préparées avec des cellules entières ou des fractions cellulaires soumises aux ultrasons. On espérait ainsi contribuer à réduire la fréquence des abcès hépatiques chez les bovins de boucherie. On utilisa à cette fin 108 veaux que l'on répartit en cinq groupes, de la façon suivante: I - témoins non inoculés; II - témoins ne recevant que l'adjuvant; III - sujets recevant 15.5 mg de protéine d'un toxoïde à base de cellules fragmentées et soumises aux ultrasons; IV - sujets recevant 10.5 mg de protéine d'une toxoïde cytoplasmique; V - sujets recevant 15.5 mg de protéine d'une toxoïde cytoplasmique. On plaça tous ces veaux dans des conditions correspondant à celles qui prévalent dans les parcs d'engraissement de l'Alberta et on examina leur foie, lors de l'abattage. Le résultat le plus prometteur accompagna l'injection de 15.5 mg de protéine d'une toxoïde cytoplasmique. Les sujets de ce groupe ne présentèrent pas de lésions hépatiques cicatrisées et la fréquence des abcès hépatiques n'y atteignit que 10%, comparativement à une moyenne de 35% (abcès et cicatrices) observée chez les sujets des groupes témoins non inoculés ou n'ayant reçu que l'adjuvant. La toxoïde préparée à l'aide de cellules entières soumises aux ultrasons ne réduisit pas la fréquence des abcès hépatiques. Ces résultats laissent supposer que l'immunisation pourrait réduire la fréquence des abcès hépatiques chez les bouvillons des parcs d'engraissement.

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INTRODUCTION

Liver abscesses which develop in feedlot cattle represent an economically important disease caused in the greatest percentage of cases by *Sphaerophorus necrophorus*. In the western provinces of Canada where the beef cattle population is concentrated and the feedlot system of finishing cattle is a common practice the incidence of liver abscesses may average about 30-40% of slaughtered animals. It is believed that ulcerated lesions of the rumen and other organs of the digestive tract are produced by the feeding of coarse grain with little or no roughage and that *S. necrophorus* is the underlying bacterial cause (5). The organism is thought to enter the portal circulation when there is an inflammation and necrosis localized in these organs of the digestive tract. The post mortem findings consist of single to multiple, small or large abscesses in cattle being fattened in feedlots (5). Other species of bacteria such as *Streptococcus* spp and *Corynebacterium pyogenes* may be associated with *S. necrophorus* in some of the abscesses (2, 8).

There is no published report on the protective action of vaccines prepared from *S. necrophorus* against the infection of bovine livers by this organism. This study was carried out to evaluate the efficacy of immunizing cattle with toxoids of *S. necrophorus* sonicated whole cells and cell fractions to prevent or reduce the incidence of bovine liver abscesses.

MATERIALS AND METHODS

Strain

S. necrophorus strain designated as LA 19 was used in this study. The organism was isolated from a bovine liver abscess and identified by procedures used in a previous study (4).

Preparation of Sonicated and Cytoplasmic Toxoids

The cells were grown in bulk by the method described by Garcia and McKay (3). The sedimented cells were washed twice in saline (0.85% NaCl). The sediment was resuspended to a concentration of 20 mg/ml of dry weight in saline. Cells were ruptured ultrasonically for 18-20 min in a MSE 100 sonic vibrator. An almost complete disruption of the cells by this technique was observed by phase microscopy. The sonicated cells were centrifuged at 18,000 X g for 15 min and separated into the supernatant and sediment. The supernatant was considered to consist of the intracellular or cytoplasmic fraction and was designated as such. The sediment containing the crude cell walls was set aside for studies on *S. necrophorus* endotoxin.

Protein determinations were made of each fraction by the method of Lowry *et al* (7). The cytoplasmic and the sonicated cell fractions contained 5.0 mg/ml and 9.5 mg/ml protein respectively.

The toxoids used in vaccination were made from the sonicated, unfractionated cells (sonicated toxoid) and the cytoplasmic fraction (cytoplasmic toxoid). The latter fraction was relatively free of crude cell wall materials. Both preparations were treated with 0.06 M formaldehyde in the presence of 0.025 M lysine and incubated at 25°C for two to four weeks. Portions of these toxoided antigens were precipitated with 10% aluminum-potassium sulfate while maintained at pH 5.5 following the procedure of Kawamura (6). A previous study (1) involving various adjuvants indicated that alum precipitated *S. necrophorus* antigens elicited the highest serological response. The final concentration of the alum precipitated antigen suspension was adjusted to 1 mg/ml protein. Prior to field tests, the various preparations were injected subcutaneously in the neck of several calves in doses ranging from 1.0-20.0 ml. The larger doses produced severe reactions at the site of injection resulting in hot tender swellings. Doses of 5.0 and 10.0 ml produced smaller lumps which were found to dissipate in a few weeks and were not considered significant at slaughter by the meat inspector.

Immunization Procedures

In May of 1972, 108 beef animals approximately 500-600 lbs and composed of 76 steers and 32 heifers were purchased at auction from several owners at Lethbridge, Alberta. The calves were placed on pasture and randomly divided into five groups and treated as follows: I. uninoculated control

— 32 animals, II. adjuvant control — 17 animals, III. sonicated cell toxoid — 19 animals (total dose 15.5 mg protein), IV. cytoplasmic toxoid — 20 animals (total dose 10.5 mg protein) and V. cytoplasmic toxoid — 20 animals (total dose 15.5 mg protein). All animals were ear tagged with a distinctive color for each group and a separate number for each animal. The animals were bled and the sera used for the serological tests. The actual dosage for each group is included in Table I. On June 26, 1972, Groups III, IV and V were given an initial dose of toxoids subcutaneously in the neck region. Seventeen of the 49 control animals (Group II) received 10.0 ml of aluminum base adjuvant only (adjuvant control). On August 22, eight weeks later, a booster dose was given to the toxoid inoculated groups. The animals were placed in the feedlot and introduced to a high energy ration made up largely of grain without antibiotics. The grain ration consisted of 90% steam rolled barley, 5% beet pulp and 5% plain concentrate (32% protein without D.E.S.). The grain to roughage ratio was 9:1. On October 17, 16 weeks after the initial injection while in the feedlot, the toxoid inoculated groups were given a second booster dose of 0.1 mg/ml protein in 5.0 ml of saline. Sera were collected for serological tests prior to each injection. At various intervals the animals were observed for clinical signs of ill health. The animals were killed in December 1972 and January 1973 upon reaching 1000-1100 lbs live weight. All livers were inspected at slaughter and the numbers of scars and abscesses were noted. A few abscessed livers randomly chosen from the slaughtered animals were subjected to bacteriological examination. All were positive for the presence of *S. necrophorus*.

Serology

Immune response was assessed by a double immunodiffusion technique on 1% ionagar plates containing borate buffer (pH 8.0). Six peripheral wells, 5 mm in diameter were spaced 6 mm from the centre well. The titration wells were arranged so that each plate would contain titrated antigen or antibody (two-fold dilutions to 1/16) surrounding the centre well containing the homologous undiluted antibody or antigen. The undiluted antigen contained 4.2 mg/ml cytoplasmic protein. The plates were incubated at 25°C in a humid chamber for four days. Negative reactions were rechecked for seven days. Distinct precipitation lines were considered as a positive indication of immune response.

Statistical Analysis

Data were subjected to statistical analysis using the single classification analysis of variance (10).

RESULTS

The results of the experiments using the various toxoids are recorded in Table I. Thirty-five percent of the livers in the control group including the adjuvant control showed lesions, of which 27% were abscesses and 8% were healed lesions (scars). An interesting observation is the lower incidence of liver abscesses and scars in the adjuvant control group (29%) compared to 38% in the uninoculated controls. The livers of animals vaccinated with the sonicated toxoid had an incidence of 37% abscesses and scars. The cytoplasmic toxoid group receiving an initial dose of 5.0 mg protein followed by a second dose of 5.0 mg and a final dose of 0.5 mg showed a slight reduction (30%) in the incidence of abscesses and scars. However the average number of abscesses per infected liver was reduced to 1.4 compared to 6.5-7.0 in the sonicated toxoid and control groups. The most promising result was obtained with the injection of cytoplasmic toxoid containing 10.0 mg protein in the initial dose and followed with doses of 5.0 mg and 0.5 mg. Liver abscess incidence was markedly reduced to 10% in this group. This reduction was statistically significant ($P<.05$) compared to the uninoculated control. Moreover, no scars were found in the livers and the average number of abscesses per liver (2.0) was low. The significant decrease in the abscesses in the latter group and the decrease in the number of scars in both cytoplasmic treated groups constituted the main features of this experiment.

The results of the agar-gel immunodiffusion tests are listed in Table II. Most of the positive sera produced strong precipitin lines with the undiluted antigen and the half antigen dilution. None of the pre-

inoculation sera showed precipitin lines with the cytoplasmic antigen. When the sera were tested eight weeks following the initial dose of the toxoids, 35% of the sera of control animals not receiving toxoid showed precipitin lines against the antigen. The sera collected from the sonicated toxoid group showed 74% with lines, 70% in the cytoplasmic toxoid (smaller dose) and 95% with the larger dose. Following the second and third doses of the toxoids the number of animals showing precipitin lines dropped to 31% in the controls and to 35-42% in the toxoid groups. Seventy-five percent of animals with liver abscesses had sera which were negative for precipitins on the last bleeding date (October 16) prior to slaughter.

DISCUSSION

Results of this study suggest that cattle may be protected against liver abscesses with the use of *S. necrophorus* cell extracts. The cytoplasmic toxoid produced the most promising results. The amount of cytoplasmic toxoid injected appeared to be critical. For instance, the group receiving an initial dose of 10.0 mg protein and a total of 15.5 mg in the three doses had abscesses in only 10% of the livers without scars compared with 35% liver abscesses with scars in the control groups. Whereas in the group receiving a smaller dose of cytoplasmic protein (10.5 mg total) the abscess incidence was just slightly reduced to 30% and one scar was found. Smith and Jones (9) indicated that nonparasitic induced scars result from the healing of abscesses caused in most instances by *S. necrophorus*. The number of abscesses per infected liver in the cytoplasmic toxoid groups also was reduced markedly.

The inoculation of sonicated toxoid did not reduce the liver abscess incidence probably because insufficient cytoplasmic antigen was present to confer the same level of immunity or protection in animals as that produced by the cytoplasmic toxoid. Indeed, all animals with liver abscesses in this group had sera that were negative

TABLE I. Results of Experiments with *S. necrophorus* Toxoids

Group	Treatment	No. of Animals	Initial Dose (ml)	Total Dose[a] (mg protein)	Abscessed Livers No.	Abscessed Livers %	Fibrous[b] Scars No.	Fibrous[b] Scars %	Abscess and Scars No.	Abscess and Scars %	Abscesses per Infected Liver Ave. No.
I	Control (uninoculated)	32	0.0	0	10	32	2	6	12	38	7
II	Adjuvant Control[c]	17	10.0	0	3	18	2	12	5	29	6
	Total Controls	49			13	27	4	8	17	35	6.5
III	Sonicated Toxoid[d]	19	10.0	15.5	5	26	2	11	7	37	7.0
IV	Cytoplasmic Toxoid[e]	20	5.0	10.5	5	25	1	5	6	30	1.4
V	Cytoplasmic Toxoid	20	10.0	15.5	2	10	0	0	2	10	2.0

[a]Total Dose: Initial dose of toxoid (1 mg/ml protein) + 5.0 mg protein toxoid (8 weeks later) + 0.5 mg protein toxoid (16 weeks after initial dose). All doses given subcutaneously
[b]Healed abscess, not considered parasitic (9)
[c]10% aluminum-potassium sulfate, injected subcutaneously
[d]Whole organism disrupted ultrasonically and formalized, alum precipitated
[e]Crude bacterial cell extract minus the cell walls, alum precipitated

TABLE II. Results of the Agar-gel Immunodiffusion Tests

Group	Treatment	Preinoculation Sera Positive/Total	Preinoculation Sera %	Postinoculation Sera Positive/Total	Postinoculation Sera %
I.....	Uninoculated control	0/32	0	9/32[a]	28
II.....	Adjuvant control	0/17	0	8/17	47
	Total Controls	0/49	0	17/49	35.0
III.....	Sonicated Toxoid	0/19	0	14/19	74.0
IV.....	Cytoplasmic Toxoid	0/20	0	14/20	70.0
V.....	Cytoplasmic Toxoid	0/20	0	19/20	95.0

[a]Serum from uninoculated animals

for precipitins on the October 16 bleeding. Fewer abscesses developed in the control group inoculated with the alum adjuvant alone. There is at present no clear explanation for this since the sonicated toxoid, which also contained this adjuvant, did not provide any detectable protection.

It would be highly desirable to produce an efficient vaccine against *S. necrophorus* infections particularly bovine liver abscesses. Evaluation of the efficacy of such a vaccine is particularly difficult because it is not easy to reproduce consistently hepatic lesions by experimental inoculation of *S. necrophorus* (5). Moreover, the intraportal route may not be the sole mode of infection under natural conditions. Thus large groups of animals maintained under field conditions are required to obtain convincing evidence that vaccination is useful in the prevention of this disease. Since there is some indication that the amount of protein antigen given may be related to the degree of immunity produced experiments are now in progress using a larger number of cattle vaccinated with a more purified toxoid of cytoplasmic protein.

It must be emphasized that these results are preliminary and that much more data are required before a satisfactory toxoid may be devised to prevent this disease. The number of doses required, the frequency of administration, the amount of protein antigen in each dose, the type of antigen (mono- or polyvalent) most suitable and the age at which the calves should be vaccinated are only a few of the basic questions that must be answered by experimental trials. However, a start has been made along lines which, as far as we are aware, have not been attempted with success in the past.

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The relationship between egg and worm counts for *Nematodirus* may be of less value since 12 BZ treated animals had zero egg counts with adult worm counts from 0 to 1840. Black (1964) and Kingsbury (1965) similarly reported difficulty in measuring of *Nematodirus* egg counts in young animals, while McKenna (1981) found *Nematodirus* counts of up to 400 worms associated with egg counts below the detectable level of 100/g faeces.

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Cultural characteristics and virulence of strains of *Fusobacterium necrophorum* isolated from the feet of cattle and sheep

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SUMMARY: Sixty-one isolates of *Fusobacterium necrophorum* were recovered for study. Thirty-one were obtained from lesions of foot abscess in cattle (25) and sheep (6), 28 were from interdigital lesions in cattle and 2 were from the normal interdigital skin of cattle. The majority of isolates from lesions of foot abscess were virulent, belonged to biotype AB (Fievez 1963), produced flat, irregular shaped, greyish colonies and haemolysis on blood agar, and grew as turbid filamentous suspensions in liquid media. They produced a soluble exotoxin, a leucocidin, and were pathogenic for cattle and mice. Virulent isolates also produced a haemolysin which most readily lysed bovine, equine and chicken erythrocytes; those from sheep were less susceptible while those of rabbit and pig were the most resistant. Isolates recovered from lesions of the feet not classified as foot abscess and from clinically normal feet were predominantly of the B biotype and caused few experimental lesions, produced convex, round, yellow colonies, flocculated and sedimented while growing in liquid medium and produced little or no haemolysin or leucocidin. Routine differentiation between virulent and non-virulent bovine isolates of *F. necrophorum* could be achieved by assessing the colour, morphology, and degree of haemolytic activity of colonies grown on blood agar.
Aust. vet. J. 62: 43-46

Introduction

In ruminants, diseases caused by the Gram-negative anaerobic rod *Fusobacterium necrophorum,* include foot abscess, bovine liver abscess and calf diphtheria (Langworth 1977). Several studies have reported its recovery from greater than 80% of bovine liver abscesses where 2 distinct biotypes, designated A and B, and an intermediate biotype, AB have been described (Fievez 1963; Kanoe *et al* 1978; Berg and Scanlan 1982; Scanlan and Hathcock 1983). Strains of biotypes A or AB were recovered more consistently from abscesses than were strains of biotype B, and the reciprocal situation was found for isolates from rumenal contents (Kanoe *et al* 1978; Berg and Scanlan 1982).

On blood agar, type A colonies appear flat, smooth, irregular in outline, metallic grey in colour and are haemolytic, whereas type B colonies are convex, regular in outline, yellow in colour and are less haemolytic than type A strains (Fievez 1963). Type A strains also grow as turbid suspensions containing predominantly filaments in liquid media while type B strains grow as short cells which sediment (Fievez 1963). Biotype AB colonies are of intermediate appearance (Fievez 1963).

Isolates of *F. necrophorum* have been recovered from lesions of ovine foot abscess (Roberts 1967) but their cultural characteristics have not been examined closely. Recent studies on the pathogenesis of bovine foot abscess have indicated that subcutaneous inoculation of *F. necrophorum* through the interdigital skin of cattle produces typical foot abscess from which the organism can be recovered readily (Clark *et al* 1984). Younger cultures (18h) contain a greater proportion of filaments, produce higher titres of leucocidin than older cultures (Emery *et al* 1984) and are more pathogenic (Clark *et al* 1984). In order to identify determinants of virulence in *F. necrophorum* the present study investigated strains of the bacterium isolated from the normal feet of cattle, and from lesions of foot abscess in cattle and sheep for their virulence, cultural characteristics, and production of leucocidin and haemolysin.

Materials and Methods

Isolation of F. necrophorum

Swabs taken from lesions of foot abscess, interdigital dermatitis or from normal interdigital skin of cattle were placed into SBL transport medium pH 7.2 (Clark *et al* 1972). Within 3 h, material collected by the swab was diluted 10^{-2}, 10^{-4} and 10^{-6} using sterile broth* and 0.1 ml quantities of these dilutions distributed over the surfaces of enriched brain-heart-infusion blood agar (BHIBA) plates (Berg and Loan 1975) that contained 5% haemolysed bovine blood. The plates were incubated at 37°C in an atmosphere of 90% H_2 and 10% CO_2 and examined at 3 and 5 days for presence of colonies of *F. necrophorum*. Subsequently, colonies were passaged on the same media. For growth in liquid media, colonies from agar plates were inoculated into Eugon broth (EB) pH 8.2* containing 2g/l yeast extract†, and cultured anaerobically for 18h or 3 days at 37°C. The relative proportions of filaments and short cells were assessed and bacteria were counted as described previously (Emery *et al* 1984).

Identification of F. necrophorum

After growing anaerobically on BHIBA, 61 strains of *F. necrophorum* were identified according to the characteristics detailed by Fievez (1963) and biochemical tests described by Moore and Holdeman (1974). Criteria in their identification included: colonial morphology from which the colour and appearance of colonies allowed classification into biotypes A, AB and B (Fievez 1963); fetid odour, Gram-negative staining; production of indole; and haemolysis on blood agar plates. *F. necrophorum* was differentiated from other fuso-bacteria by its production of lipase when tested on egg-yolk agar.

Examination of Haemolytic and Leucocidal Activity

The haemolytic activity of strains of *F. necrophorum* grown on solid media was determined after incorporation of 5% intact erythrocytes from different species into Eugon agar containing 0.2% yeast extract†. After incubation for 4 or 7 days, haemolysis was classed as β-type (clear) or α-type (greenish in colour with partial haemolysis), and the zone of haemolysis was measured from the edge of the colony.

Supernates from ten 18h liquid cultures of strains from each bio-type of *F. necrophorum* were serially diluted 2-fold in PBS in round-bottomed microtitre trays and an equal volume (100 μl) of 2% erythrocytes was added to duplicate wells before the mixture was incubated at 25°C for up to 24h.

The titre of haemolysin was taken as the reciprocal dilution of supernate causing 50% haemolysis. Tests for leucocidal activity in the same supernates were conducted as described by Emery *et al* 1984.

Comparisons of Virulence of F. necrophorum *Isolates*

Liquid cultures (18 h) of 4 isolates of *F. necrophorum*, S196, V93G (biotypes AB) and V91Y, V93C (biotypes B) were each diluted in EB to contain the equivalent of (a) $2x10^9$ or (b) $2x10^7$ short cells per ml. Each strain was injected intraperitoneally into mice. Groups of 6 mice received 0.25 ml of either (a) or (b). The mice were observed for 14 days for the development of abscesses and were examined at necropsy to determine the presence of lesions and for recovery of the inoculated bacterium.

In a second experiment, 6 Hereford calves aged 10 months were sedated with Rompun®‡ and one foot of each calf was inoculated subcutaneously with one ml of EB containing $5x10^8$ cells of isolate S196 while another foot of each calf was inoculated with $5x10^8$ V93C. The calves were examined weekly for 4 weeks for signs of lameness and foot abscess.

Results

Cultural Characteristics of F. necrophorum *Isolated from Bovine and Ovine Feet*

Sixty-one isolates of *F. necrophorum* from the feet of cattle and sheep were identified, 30 being classified as biotype AB and 31 as biotype B by their growth on BHIBA (Table 1). No classical biotype A colonies of reflective, metallic grey colour were observed. The 2 isolates recovered from normal feet of cattle were of biotype B and the majority of those (22 of 28) isolated from interdigital lesions of cattle (but not causing foot abscess) were also of this biotype. In contrast, the majority of isolates (24 of 31) recovered from lesions of foot abscess in sheep and cattle were of biotype AB.

Regardless of the origin of each isolate, strains of biotype AB produced β-type (complete) haemolysis of bovine blood in agar, and grew in EB as turbid suspensions containing predominantly filaments (Table 1). By comparison, strains of biotype B produced α-type (partial) haemolysis on bovine blood agar, and grew in EB as short cells which flocculated and sedimented rapidly after resuspension.

Supernates from 18h liquid cultures of biotype AB strains of *F. necrophorum*, regardless of their origin, contained haemolytic and leucocidal activity against bovine erythrocytes and peripheral blood leucocytes, respectively. Titres of haemolysin ranged from 4 to 2048, while the titres of leucocidin ranged between 480 and 14,400, (Table 1). In contrast, strains of biotype B consistently failed to produce haemolysin titres greater than 4, or leucocidin titres greater than 200 (Table 1).

Haemolysis of Erythrocytes from Different Species

The spectrum of haemolysis either in suspensions or in blood agar prepared from the erythrocytes of cattle, sheep, horses, chickens, rabbits and pigs by strains of *F. necrophorum*, is shown in Table 2. On blood agar, 3 strains of biotype B partially haemolysed cattle, sheep, horse and rabbit erythrocytes, and completely lysed chicken and pig erythrocytes. Bovine and ovine strains of *F. necrophorum* of biotype AB consistently lysed erythrocytes from all 6 species; the haemolysis was complete for all except rabbit cells (although one bovine strain exhibited β-type haemolysis of horse erythrocytes) (Table 2). Examination of the extent of the zone of haemolysis suggested that chicken and rabbit erythrocytes were the most sensitive, with those of pigs, cattle, sheep and horses following in order of increasing resistance.

Supernates from 18h EB cultures of 3 strains of biotype B did not contain detectable haemolytic activity against erythrocytes in suspension from any of the species examined. Comparable supernates from 7 strains of biotype AB exhibited titres of haemolysin against the 6 species of erythrocytes ranging from 0 to greater than 256. An examination of the respective titres in Table 2 revealed that suspended erythrocytes of cattle, horses and chickens showed comparable and

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* Eugon broth, Becton Dickinson and Co, Cockeysville, Maryland, United States of America
† Difco Labs, Detroit, Michigan, United States of America
‡ Bayer Australia Ltd, Botany, New South Wales

TABLE 1

Cultural characteristics of strains of F. necrophorum

Origin of strain (animal)	No. of strains	Biotype (number of strains)	Haemolysis in blood agar*	Growth in EB	Predominant morphology in EB	Leucocidin titre (range)*	Haemolysin titre (range)*
Normal feet (cattle)	2	AB (0)	—	—	—	—	—
		B (2)	α	short cells	floc† sed	<10	≤ 2
Interdigital lesions (cattle)	28	AB (6)	β	filaments short cells	turbid	860-6,400	16-1,024
		B (22)	α		floc/ sed	0-160	≤2
Foot abscess (cattle)	24	AB (18)	β	filaments short cells	turbid	480-14,400	4-2,048
		B (7)	α		floc/ sed	0-88	≤2
Foot abscess (sheep)	8	AB (8)	β	filaments	turbid	1,250-8,250	16->256
		B (0)	—	—	—	—	—

*Haemolysis, leucocidin and haemolysin tests were performed using bovine cells.
†floc/sed: flocculating and sedimenting growth; EB: Eugon broth.

greatest sensitivity to haemolysis, sheep cells were slightly less sensitive, and rabbit and pig erythrocytes were the least susceptible. The titres for haemolysin were generally 5 to 20% of those for leucocidal activity. One isolate, S196, consistently produced low titres of haemolysin in 18h EB supernates, but generated leucocidin titres of greater than 1000.

Comparative Virulence of 4 Strains of F. necrophorum

Of the 6 feet inoculated with $5x10^8$ cells of strain S196 (biotype AB) lameness and abscesses were produced in all, and 5 of the abscesses discharged. A similar inoculum of $5x10^8$ V93C (biotype B) produced slight swelling in 3 of 6 feet, all of which resolved without abscessation. Both AB isolates, V93G and S196 proved highly toxic for mice at a dose rate of $5x10^8$ cells per mouse, killing all 6 mice, in each respective group. At the same dose, V93C and V91Y (biotypes B) killed 3 of 6 and 4 of 6 mice, respectively. There was a tendency for mice inoculated with the AB biotypes to die within the first week after inoculation, while those inoculated with the B biotypes died at intervals over the ensuing 14 days. A reduction of 100-fold in the inoculum resulted in 2 deaths in each group of 6 mice inoculated with the AB strains, while all of the mice given $5x10^6$ cells of either V93C or V91Y strains survived. At necropsy, abscesses were present in the abdominal cavity of 5 of the 8 mice that survived after receipt of the biotype AB strains, but in only 4 of the 17 mice which survived after receiving the biotype B strains.

Discussion

Examination of isolates of *F. necrophorum* isolated from the feet of cattle and sheep revealed that strains associated with lesions of foot abscess were predominantly of biotype

TABLE 2

Haemolytic activity of strains of *F. necrophorum*

Origin of strain (animal)	No. of strains tested	Biotype	Erythrocytes examined (cm radius of haemolysis)									
			Cattle		Sheep		Horse		Chicken		Rabbit		Pig	
Haemolysis of Blood Agar												
Normal Feet (cattle)	2	B	α	(<0.1)	α	(<0.1)	α	(<0.1)	β	(0.5)	α	(1.0)	β	(0.7)
Foot abscess (cattle)	8	AB	β	(0.4)	β	(0.4)	β	(0.3)*	β	(1.0)	α	(1.0)	β	(0.7)
	1	B	α	(<0.1)	α	(<0.1)	α	(<0.1)		n.t.†		n.t.		n.t.
Foot abscess (sheep)	8	AB	β	(0.4)	β	(0.4)	β	(0.3)	β	(1.0)	α	(1.0)	β	(0.7)
B. Haemolysis of Erythrocyte Suspensions												
Normal Feet (cattle)	2 — M53	B	—		—		—		—		—		—	
	— V93C	B	—		—		—		—		—		—	
Foot abscess (cattle)	2 — V93G	AB	128		64		128		16		8		16	
	— S349	AB	—		—		2		32		—		—	
	1 — V91Y	B	—		—		—		—		—		—	
Foot abscess (sheep)	5 — Nec 3	AB	4		—		4		4		—		—	
	— Nec 14	AB	>256		>256		>256		>256		64		32	
	— Nec 18	AB	>256		>256		>256		>256		64		32	
	— Nec 150	AB	16		4		16		16		—		16	
	— SJ83	AB	>256		>256		>256		>256		128		64	

Results are expressed as the reciprocal of the dilution causing 50% haemolysis of a 1% erythrocyte suspension.
*One strain produced α-type haemolysis against horse erythrocytes.
†n.t. — not tested

AB. These produced grey colonies on blood agar and were virulent for cattle and mice. In liquid media they produced turbid filamentous growth, and generated haemolysin and leucocidin. No definite biotype A strains were isolated. In contrast, 2 isolates from normal feet and the majority of those recovered from interdigital lesions of bovine feet were of biotype B, producing yellow or cream colonies on blood agar, sedimenting growth in liquid media, and were relatively avirulent for cattle and mice. The same characteristics have been described for strians of *F. necrophorum* isolated from rumenal contents and from lesions of rumenitis and liver abscess in feedlot cattle (Fievez 1963; Berg and Scanlan 1982; Scanlan and Hathcock 1983). Strains isolated from lesions were generally of biotypes A and AB, whereas those in the rumenal contents were of biotype B (Kanoe *et al* 1978; Scanlan and Hathcock 1983).

Haemagglutination of chicken erythrocytes has been used to distinguish *F. necrophorum* biotypes A and AB from those of biotype B (Beerens 1954; Fievez 1963; Berg and Scanlan 1982). Fievez (1963), also routinely distinguished between these biotypes by the colour, morphology and haemolytic activity of colonies grown on equine blood agar. Similarly, in our study, isolates classified as virulent biotype AB from the feet of cattle could be differentiated from those of B biotype by colour, morphology and haemolytic activity of colonies grown on bovine blood agar. Although growth in EB was also characteristic for each biotype, the generation of leucocidin was the most sensitive test for distinguishing between strains of *F. necrophorum* which were virulent or not for cattle. In contrast to the results of Fievez (1963), we found that ovine and bovine erythrocytes when present either in blood agar or in suspension were readily lysed by *F. necrophorum* strains of biotype AB or their supernates from liquid culture, respectively. The relative susceptibility of erythrocytes to lysis by supernates from *F. necrophorum* also paralleled the relative sensitivities of leucocytes from the same species to cytolysis by leucocidins produced by the same strains (Emery *et al* 1984).

It has been argued that trauma or devitalisation of tissue, such as that caused by rumenal acidosis or penetrating lesions of the foot is a prerequisite for colonisation by *F. necrophorum* (Scanlan and Hathcock 1983). Although virulent biotypes were not isolated from the normal interdigital skin of cattle in this study, virulent isolates of *F. necrophorum* have been recovered from the rumenal contents of cattle. Eighty-three isolates were obtained by Kanoe *et al* (1978), and 19 were of biotype A and 64 of biotype B; Berg and Scanlan (1982) recovered isolates representative of biotype A from the rumens of 9 of 12 cattle. Thus it is proposed that virulent strains of *F. necrophorum* in the environment or gastrointestinal tract initiate lesions when favourable conditions occur for their establishment. Alternatively, it has been postulated from *in vitro* studies that the 3 biotypes can mutate in the direction A → AB → B (Beerens *et al* 1971). This suggestion argues against the persistence of virulent biotypes, and apart from a decline in the titres of leucocidin and haemolysin produced after prolonged passage of virulent biotypes *in vitro* we have found no cultural evidence to support this hypothesis.

That the leucocidin is a determinant of virulence for *F. necrophorum* has been proposed from observations which correlate toxin production with the capacity of different strains to form abscesses in experimental animals (Coyle-Dennis and Lauerman 1978; Scanlan 1979; Berg and Scanlan 1982). Moreover, the endotoxins of both A and B biotypes exhibit equivalent biological potency (Berg and Scanlan, 1982). Except for one isolate (S196), the titres of leucocidin and haemolysin show a positive correlation (unpublished data) raising the possibility that both activities may be mediated by a common molecule. The association between strains of biotype AB and lesions of foot abscess in cattle implies that potential vaccines against infection should be sought from these strains of *F. necrophorum*. In this regard, protection against challenge with *F. necrophorum* biotype AB and antibodies which neutralise the leucocidin and haemolysin of *F. necrophorum* are invoked in cattle vaccinated with supernates containing leucocidal activity (unpublished data).

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Aust Vet J. 1986 Apr;63(4):107-10.

SO Veterinary Microbiology, (1996) Vol. 48, No. 1-2, pp. 1-7.

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Studies into immunisation of cattle against interdigital necrobacillosis

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SUMMARY: Calves were immunised with fractions of *Fusobacterium necrophorum* incorporated in mineral oil adjuvant and then each foot was experimentally exposed to interdigital necrobacillosis (foot abscess) by subcutaneous injection of homologous organisms through the interdigital skin. The number of cells from an 18 h liquid culture that might be expected to cause 50% of the feet of control calves to develop marked swellings following subcutaneous injection was shown to be approximately 2.2×10^2 cells. Immunity was shown to be associated with antigens that were located in the supernatant of the culture, and which may be identical with or closely associated with the exotoxins.
Aust Vet J 63: 107-110

Introduction

Vaccines against bovine interdigital necrobacillosis (IN)†, a disease caused by *Fusobacterium necrophorum*, are available, but their efficacy has not been fully established. Controlled comparative studies have not been reported, but following the use of a commercial vaccine‡ in dairy herds, Gilder (1960) found a marked reduction in the incidence of IN. The incidence of liver abscess in grain fed cattle, also caused by *F. necrophorum*, has been decreased by immunisation. Using an alum precipitated vaccine prepared from the cytoplasmic fraction of *F. necrophorum*, Garcia *et al* (1974) reduced the incidence of liver abscess from 35% in control cattle to 10% in vaccinated cattle. The mechanisms involved in immunity against *F. necrophorum* infection in cattle have not been elucidated although studies in rabbits (Roberts 1970) suggested that acquired resistance to experimental infection could be antibody mediated.

The lack of reliable methods for reproducing IN in cattle has been an impediment to the evaluaion of vaccines against the disease. However, recent research has shown that IN can be produced in cattle by injecting young cultures of toxigenic strains of *F. necrophorum* either into or through the interdigital skin (Berg and Loan 1975; Clark *et al* 1985).

We describe studies into immunisation of cattle against IN which examine the capacity of vaccines to protect calves against experimental infection of the feet.

Materials and Methods

Experimental Animals

Observations were made upon 62 Hereford calves (33 steers and 29 heifers) aged between 5 and 10 months at the CSIRO Field Station at Werribee, Victoria.

Bacterial Strain and Cultural Techniques

F. necrophorum strain FnBI (formerly NEC 196) (Clark *et al* 1985) was used. It was originally isolated from a heifer with IN, produced high titres of leucocidin (Emery *et al* 1984) and had a type AB colony (Fievez 1963).

The cells used in experiment 1 to prepare vaccines and to infect feet were grown in modified Eugonbroth§ (MEB, Claxton *et al* 1983) and those used in experiments 2 and 3 were grown in Eugonbroth containing 1% maltose, 0.5% yeast extract¶, 0.5% meat extract¶, 0.5% cysteine HCl#, haemin# 5 ug/ml and menadione# 0.5 ug/ml (MEL), following the method of Berg and Loan (1975).

All cultures were incubated at 37°C in an atmosphere of 90% H_2 and 10% CO_2, and were used after 18 h when most FnBI cells were present as filaments and the remainder as single cells (Emery *et al* 1984). Cell numbers were assessed as the number of single cells or their equivalent in filaments, per ml. Cell suspensions of known numbers per ml were prepared using counting chambers**. Titres of leucocidin in cultures were determined using the release of Cr^{51} from bovine leucocytes as described by Emery *et al* (1984).

Experimental Design and Procedures

Experiment 1 — Immunisation of calves against experimentally induced IN — Twenty-eight calves were distributed equally according to age, sex and sireline into 4 groups of 7 calves each and mineral oil adjuvant vaccines against IN were administered to calves in groups 1, 2 and 3, while those in group 4 were kept as non-vaccinated control animals. The vaccine given to group 1 contained FnBI whole culture that had been concentrated 10 times using a XM100 A membrane†† with MW retention of 100,000. Group 2 was given a vaccine that contained FnBI cytoplasmic fraction (Garcia *et al* 1974) that had been derived from concentrated, washed cells by sonication and centrifugation. The vaccine given to group 3 contained cell-free culture supernatant fluid that had been concentrated 10 times using a XM100 A membrane. The first vaccine contained 4×10^{10} unwashed cells, and the second and third vaccines contained the cytoplasmic and supernatant components respectively corresponding to 4×10^{10} cells, in each 5 ml dose. Five ml of each vaccine was given by subcutaneous injection on each of 3 occasions separated by intervals of 4 weeks. The titre of leucocidin in the components used on the 3 occasions ranged from 4,000 to 80,000 in the concentrated whole culture and culture supernatant fluid, and from 15 to 42 in the concentrated cytoplasmic fraction.

Two weeks after the third injection of vaccine, all feet were inoculated with 1.4×10^9 cells of FnBI.

Experiment 2 — Estimation of the ID50 of FnBI for calves feet — The number of FnBI cells required to produce IN in 50% of feet was investigated in 10 calves. Each calf was given one injection of each of a dilution of culture into separate feet. Administration of each dilution was equally distributed among the 4 feet available. The injected volume of 1 ml

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† Synonyms: foul-in-the-foot, foot abscess
‡ FRA Vaccine®, Commonwealth Serum Laboratories, Parkville, Victoria
§ Becton Dickinson and Co, Cockeysville, Maryland 21030, United States of America
¶ Difco Laboratories, Detroit, Michigan, United States of America
\# Sigma Chemical Company, St. Louis, Missouri, United States of America
** C A Hausser and Son, Philadelphia, United States of America
†† Amicon, Lexington, Massachusetts, United States of America

contained either 3.2×10^8 cells, 1.0×10^8 cells, 3.2×10^7 cells or 1.0×10^7 cells.

Experiment 3 — Comparison of the immunogenicity of FnBI culture supernatant fluid and washed cells — Twenty-four calves were distributed equally according to age, sex and sireline into 3 groups. The calves in the first group were given the mineral oil adjuvant vaccine containing cell-free culture supernatant fluid that had been concentrated 10 times using a XM100 A membrane. Those in the second group were given a mineral oil adjuvant vaccine containing FnBI cells that had been washed 3 times by centrifugation and then suspended in phosphate buffered saline (PBS) pH 7.2 containing 0.01% NaN_3. The calves in the third group (controls) were given the mineral oil adjuvant containing PBS as a placebo. Five ml of vaccine or placebo was given by subcutaneous injection on 2 occasions separated by an interval of 4 weeks. The vaccines either contained concentrated culture supernatant fluid corresponding to 4×10^{10} cells (first group), or 4×10^{10} killed washed cells (second group), in each 5 ml dose. The titre of leucocidin in the concentrated culture supernatant fluids used for vaccine production in experiment 3 ranged from 57,000 to 89,000 on the first and second occasions respectively. Two weeks after the last injection, each foot of every calf was inoculated with 2.2×10^8 cells of FnBI.

Mineral Oil Adjuvant Vaccines

Mineral oil* adjuvant vaccines were prepared that contained the oil and water phases in the ratio of 2 to 1, the emulsifiers mannide mono-oleate† (v/v 6.6/100) and polyoxyethylene sorbitan mono-oleate‡ (v/v 0.7/100) and the stabilisers aluminium monostearate (w/v 2.7/100) and aluminium hydroxide gel§ (v/v 12.4/100). All vaccines were kept at 4°C and used within 24 h of preparation. The site used for subcutaneous injection of vaccines was the loose skin behind the left and right shoulders.

Infection Techniques

Sedation and analgesia were achieved in the calves by the administraion of 2% w/v xylazine¶. The interdigital skin was washed clean with tap water and then wiped dry with cotton wool. Infection of feet was carried out by inoculaion of 1 ml of cell suspension through a 25 gauge needle subcutaneously to a depth of 15 mm at the centre of the interdigital space. Inoculation of calves feet was completed within 3 h of harvesting the 18 h cultures.

Examinations

Blood samples were collected from calves in experiments 1 and 3 at all times of vaccination, and of infection of feet and antibodies against FnBI in serums were titrated in an enzyme-linked immunosorbent assay (ELISA) and an agglutination assay. For the former, 10 μg protein/well of concentrated FnBI culture supernatant fluid was used as antigen and the assay was conducted as described previously (Emery *et al* 1985). For comparison, serums were also titrated in ELISA using 10 μg protein/well of concentrated Eugonbroth as antigen. Agglutination assays were conducted using cells from an 18 h liquid culture of FnBI after they had been washed and resuspended in PBS containing 0.25% (v/v) formalin. Equal volumes of diluted serum and bacterial cells (adjusted to an optical density# of 0.33 to 0.40) were combined and the assay was read after being held at room temperature for 16 h.

Examination of feet was carried out at weekly intervals for 4 to 6 weeks after infection. Calves were sedated with 2% xylazine* and the degree of swelling of soft tissues in the interdigital space and proximal to the coronet on the anterior and posterior aspects of the foot, was assessed on each occasion and scored as either marked, moderate, slight or nil. The presence of fistulae and discharge of pus was not taken into account in assessment because the method of infection can produce these signs in resistant animals in the absence of swelling of adjacent soft tissue. In view of the similarity between feet affected with marked swellings and those naturally affected with IN, the proportion of feet observed to have marked swellings was used for comparison of the relative susceptibility of groups of calves to IN. The protection afforded by vaccines was then calculated using the following formula.

$$\text{Percentage protection} = \frac{\text{The difference between proportions of affected feet in vaccinated and control calves} \times 100}{\text{Proportion of affected feet in control calves}}$$

At the conclusion of the experiment, calves with persistent swellings were given intramuscular injections of an aqueous suspension of procaine penicillin and dihydrostreptomycin sulphate*.

* Marcol 52 Esso Australia Ltd, Melbourne, Victoria
† Arlacel A®, Atlas Powder Co, Wilmington, Delaware, United States of America
§ Alhydrogel, Superfos, Copenhagen, Denmark
¶ Rompun, Bayer Australia Limited, Botany, N.S.W.
# Spectronic 20, Bausch and Lomb, Rochester, New York, United States of America
* Streptopen, Glaxo Australia Pty Ltd, Boronia, Victoria

Results

Experiment 1 — Immunisation of Calves against Experimentally Induced IN

Examination of the feet of all calves on 4 occasions showed that 18 of 28 (64.3%) feet in group 1, 21 of 28 (75%) feet in group 2, 15 of 28 (53.6%) feet in group 3 and 24 of 28 (85.7%) feet in group 4 were affected with marked swellings on one or more occasions (Table 1). Even when allowance was made for overdispersion, there were significant differences between the proportions of affected feet in groups 1 or 3 and that in group 4 ($p < 0.05$), but the difference between groups 2 and 4 was not significant. Variables such as age, sex and sireline of the calves did not affect these results. While there was no difference between the proportions of affected front and hind feet, the latter tended to take a longer time to develop swellings after infection. The average duration of the marked swellings in affected feet in groups 1, 2, 3 and 4 were 1.3, 1.6, 1.3 and 1.8 weeks respectively, indicating that feet of calves in groups 1 and 3 tended to remain swollen for shorter periods than those in groups 2 and 4.

The relative serological responses of calves to vaccines containing either concentrated whole culture, or concentrated cytoplasmic fraction, or concentrated cell-free culture supernatant fluid as measured by the ELISA and agglutination assay are shown in Figure 1. The antibody response detected by the ELISA assay was greatest to the concentrated culture supernatant fluid and least to the concentrated cytoplasmic fraction, while that detected by the agglutination assay was greatest to the latter and least to the former. The antibody responses to the concentrated whole culture vaccine were intermediate in both assays.

Experiment 2 — Estimation of the ID50 of FnBI for Calves Feet

The subcutaneous injection of either 1×10^7 or 3.2×10^7 cells into the feet of calves failed to produce any evidence of infection. The subcutaneous injection of 1×10^8 cells and 3.2×10^8 cells caused 1 of 10 feet, and 7 of 10 feet respectively to develop marked swellings. The 3 other feet that received 3.2×10^8 cells developed either a moderate (2) or slight swelling, while one other foot that received 1×10^8 cells also developed a slight swelling. From this data it was calculated (Thompson 1947) that the number of cells from an 18 h liquid culture of FnBI that might be expected to cause 50% of the feet of calves to develop marked swellings following subcutaneous injection was approximately 2.2×10^8 cells.

Experiment 3 — Comparison of the Immunogenicity of FnBI Culture Supernate and Washed Cells

Following the injection of 2.2×10^8 cells of FnBI subcutaneously into each foot of the 24 calves, weekly examinations

TABLE 1
Incidence of interdigital necrobacillosis (IN) in immunised and control calves following subcutaneous injection of *Fusobacterium necrophorum* strain FnBI into feet

Group	No. of calves in group	Treatment	Infection		
			Cells* injected per foot	No. of feet injected	No. of feet affected with IN†
Experiment 1					
1	7	Immunised with concentrated FnBI whole culture	1.4×10^9	28	18
2	7	Immunised with concentrated FnBI cytoplasmic fraction	1.4×10^9	28	21
3	7	Immunised with concentrated FnBI culture supernatant fluid	1.4×10^9	28	15
4	7	Non-immunised controls	1.4×10^9	28	24
Experiment 3					
1	8	Immunised with concentrated FnBI culture supernatant fluid	2.2×10^9	32	6
2	8	Immunised with concentrated killed washed FnBI cells	2.2×10^9	32	11
3	8	Non-immunised controls	2.2×10^9	32	15

* Cell numbers assessed as numbers of single cells, or their equivalent in filaments.
† Marked swellings present on at least one occasion.

for 6 weeks found that 6 of 32 (18.8%) feet in group 1, 11 of 32 (34.4%) feet in group 2 and 15 of 32 (46.9%) feet in group 3 were affected with marked swellings on one or more occasions (Table 1). There was a significant difference between the proportions of affected feet in groups 1 and 3 ($p < 0.025$) but not between those in groups 2 and 3. The age, sex and sireline of calves and location of leg (front vs hind), did not affect the results but it was observed that swellings required a longer time to become apparent in hind compared with front feet.

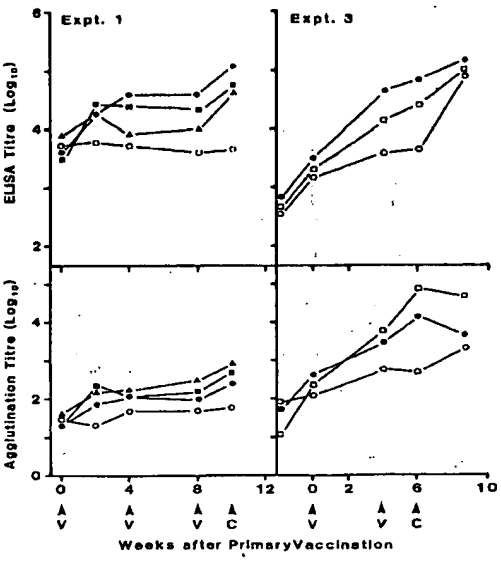

Figure 1. Mean serum antibody responses of calves following administration of mineral oil adjuvant vaccines containing FnBI culture fractions.

Experiment 1
Concentrated whole culture (■)
Concentrated cytoplasmic fraction (▲)
Concentrated culture supernatant fluid (•)
Non-immunised controls (o)

Experiment 3
Concentrated culture supernatant fluid (•)
Concentrated killed washed cells (□)
Non-immunised controls (o)
V indicates time of vaccination
C indicates time of challenge

The relative serological responses of calves to the 2 vaccines used in experiment 3 are shown in Figure 1 and indicate that antibody responses measured by the ELISA were greatest to the vaccine containing the concentrated culture supernatant fluid whereas those measured by the agglutination assay were greatest to the vaccine containing the concentrated killed washed cells.

Discussion

Typical lesions of IN can be produced in cattle by injecting 18h cultures of pathogenic strains of *F. necrophorum* through the interdigital skin (Clark *et al* 1985). In experiment 1, the arbitrarily selected infective dose of 1.4×10^9 cells produced a high incidence of affected feet in the control calves (24/28). In spite of the obvious severity of the challenge, a degree of protection against IN was obtained in groups 1 and 3 that were given vaccines containing concentrated whole culture, and concentrated culture supernatant fluid respectively. The protection afforded by these 2 vaccines in experiment 1 was assessed as 25% and 37.5% respectively. In comparison, the vaccine containing the concentrated cytoplasmic fraction from FnBI gave little or no protection. The concentrated whole culture and culture supernatant fluid both contained the membrane and secreted antigens of FnBI such as exotoxins, capsular antigens, lipopolysaccarides and outer membrane proteins, and the concentrated whole culture also contained the cell bound and insoluble antigens. While the cytoplasmic fractions contained soluble and smaller particulate antigens that were not pelleted by centrifugation at 35,000g, exotoxins were present in relatively small amounts (Emery *et al* 1984). However, Garcia *et al* (1975) described a strain of *F. necrophorum* obtained from a bovine liver abscess that possessed 2 cell bound toxins but produced no exotoxin. The cytoplasmic fraction from this strain contained an effective immunogen for cattle (Garcia *et al* 1974). It is possible that the reason for the difference between the immunogenicity of the FnBI cytoplasmic fraction and that produced by Garcia *et al* (1974) was that their strain had limited capacity to excrete toxic and protective factors from the cell.

In experiment 2, the ID50 of FnBI for calves feet was estimated to be approximately 2.2×10^8 cells. This infective dose was used in experiment 3 in order to reduce the severity of challenge below that used in experiment 1, and to facilitate the recognition of any differences in susceptibility between groups of calves.

In experiment 3, the proportion of affected feet in the control group was 15 of 32 (46.9%) and the degree of protection afforded by the vaccine containing the concentrated culture supernatant fluid was calculated to be 60%. Although

there was no significant difference between the proportions of affected feet in the control calves and those that received the vaccine containing killed washed cells, the degree of protection attributable to the vaccine was 26.7%.

Conjointly, the results obtained in experiments 1 and 3 indicated that the cell-free culture supernatant fluid of FnB1 contained most of the protective activity. While the precise nature of these antigens has still to be determined, it is possible that they are identical to or closely associated with the exotoxins as proposed by Roberts (1970). Although the serological results suggested that the ELISA was more successful than the agglutination assay in detecting the antibody responses to the cell-free culture supernatant fluid of FnB1, the ELISA used both soluble and membrane antigens and results do not indicate the specificities of the antibody produced. The encouraging results obtained in these preliminary experiments indicate that further research is warranted to identify and purify the protective antigens of *F. necrophorum* and to evaluate them as immunogens in commercial vaccines.

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The prevalence of *Giardia* in dogs and cats in Perth, Western Australia

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SUMMARY: A survey of dogs and cats in the Perth metropolitan area revealed a high prevalence of *Giardia*. Overall, 21% of 333 dogs and 14% of 226 cats were infected. More dogs and cats from refuges and breeding establishments were infected than household pets, although among the latter a significant number of dogs (9%) and cats (8%) was infected. *Giardia* did not show any breed or sex predisposition but prevalence was higher in young animals. The species of *Giardia* present in dogs and cats was identified as *G. duodenalis*, which is the same as that affecting man. The potential significance of this animal reservoir of infection to man is discussed in the light of increasing evidence that *Giardia* is a zoonosis.

Aust Vet J 63: 110-112

Introduction

Giardia (Order Diplomonadida) is an intestinal flagellate protozoan found in virtually all classes of mammals throughout the world and during the last decade has gained increasing recognition as a human pathogen. It is the most common intestinal parasite of man in the United States of America and United Kingdom (Anon. 1978a, b) and the disease is now included in the World Health Organisation Diarrhoeal Disease Control Program (Wilson 1984). In Australia, *Giardia* is considered to be the most important parasitic disease in terms of morbidity (Boreham *et al* 1981), and is one of the most common causes of diarrhoea in children with a failure to thrive (Boreham and Shepherd 1982). The prevalence is often higher in individuals from developing countries or the lower socio-economic groups where hygienic standards are compromised. Children are frequently infected (Edmeades *et al* 1981; Boreham *et al* 1981) and there is a high prevalence in institutions such as asylums, orphanages and child-care centres (Black *et al* 1977; Keystone *et al* 1978).

Until recently, *Giardia* was considered to maintain rigid host specificity but this is now disputed since a number of workers have demonstrated experimentally that inter-species transmission can occur. At least 9 different animal species, including dogs, have been successfully infected with cysts of *Giardia* isolated from different species, including man (Grant and Woo 1978; Davies and Hibler 1979; Hewlett *et al* 1982). It is therefore possible that animals and man may infect each other and that certain animal species may constitute a significant reservoir of infection for man (Schantz 1983).

Humans are very likely to be exposed to *Giardia* of animal orgin because of the ubiquity of the parasite and a survey of the prevalence in domestic animals may identify a potential reservoir for human infection. Despite the public health significance and possible zoonotic potential of *Giardia*, there have been no previous prevalence reports of *Giardia* in domestic animals in Australia. Surveys in other countries have shown that infection with *Giardia* is common and widespread in both dogs and cats (see review by Barlough 1979; Seiler *et al* 1983), although prevalence rates vary greatly due to differences in the number of animals and type of population surveyed and the diagnostic procedures employed. The aim of the present study was to determine the prevalence of *Giardia* in dogs and cats in Perth. In order to accurately assess the level of infection, the survey was not restricted to one particular group of animals.

Materials and Methods

Dog and Cat Populations

Faecal samples were obtained from 3 different populations of dogs and cats in the Perth metropolitan area. Samples were